Effect of Sewage Sludge Amended Soil on Mineral Nutrition and Metal Accumulation on Sunflower Crop

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The aim of the present study was to evaluate the suitability of sewage sludge amendment for sunflower (Helianthus annuus L.) by assessing the biomass measurements, macroelements (N, P, K, Ca, Mg) and potential hazardous metals accumulation (Mn, Cu, Zn, Cd, Ni, Pb) at different doses of sewage sludge accompanied by mineral fertilization. Sewage sludge fertilization produces significant increase of stem and leaves mass, meanwhile significant increases of calatidium mass appear in the case of 200 kg N/ha dose. Nitrogen content in calatidium increased significant after sewage sludge fertilization with doses higher than 200 kg N/ha, and the highest potassium content was registered at 400 kg N/ha dose. Phosphorus content in calatidium presents a great variability and it is not significant influenced by fertilization with sewage sludge, being observed even a downward trend. Calcium and magnesium contents in stem and leaves increases with sewage sludge dose, the highest concentrations being observed at 500 kg N/ha dose. Sewage sludge application produces increases of manganese and copper concentrations in stems and leaves. Manganese and zinc contents found in stems and leaves are higher than in calatidium, meanwhile in copper case was registered the opposite situation. Sewage sludge fertilization produces significant increases of cadmium in stems and leaves at doses higher than 300 kg N/ha but not exceed 1 mg/kg. In small doses (100-300 kg N/ha), sludge produces an increase of nickel in stems and leaves. Lead accumulation in stems and leaves is produced when applied sewage sludge doses are higher than 300 kg N/ha. There are no concerns regarding environmental issues correlated with elevated levels of metals in sunflower plants.

Keywords: sewage sludge, heavy metals, soil, nutrients, sunflower plants

Rapid industrial development and urbanization during last decades have generated a large quantity and a great diversity of wastes. Recently, a lot of attention has been paid to sewage sludge due to its increasing amounts and to the potential uses.

Use of sewage sludge in agriculture is an efficient alternative disposal technique of waste. Even if it contains macronutrients suitable for plant nutrition [1-4], sewage sludge may contain variable levels of toxic metals [2, 5, 6] that could accumulate in vegetal products, organic pollutants [7-9] and pathogenic organisms [2, 9, 10]. This is because sewage sludge is not designed for agricultural use and it has variable and sometimes unpredictable composition [11].

Due to beneficial effects and from environmental and ecologic reasons, the sewage sludge application to agricultural soils has been investigated in many countries[4, 12, 13]. Many studies report high levels of heavy metals in soil amended with sewage sludge [13, 14].It has been proven that excessive application of sewage sludge beyond crop requirements may produce groundwater contamination by nitrates and surface waters by phosphorus [15].

Also, metal accumulation in plant tissues were found in plants grown on sludge-amended soil [14, 16].

High levels of iron, copper and zinc were recorded in the case of maize and barley grown on sludge-amended soil even if sludge application led to increased yields [17].

Morera et al. [18] found that sludge doses of 80, 130 and 160 t/ha increased the average dry weight of sunflower plants (*Helianthus annuus*L.) in comparison with those grown on un-amended soil. Also, positive effects on yield of dwarf bean [19] and growth of flax [20] have been reported at different doses of sewage sludge in the soil. Furthermore, the lead and zinc uptake of plant leaves and roots was reduced in the case of dwarf bean [19].

Sewage sludge amendment rates above 4.5 kg/m^2 through increased the yield of rice, but caused risk food chain contamination as concentrations of nickel and cadmium in rice grains were found higher than Indian safe limits (1.5 mg/kg) [16].

Application of different rates of sewage sludge in the case of mung bean (*Vignaradiata* L.) produced increments in shoot length, leaf area and total biomass. Also, these treatments lead to high levels of nutrients (N, K, Ca, Mg) and heavy metals (Cu, Cd, Zn, Mn, Ni, Cr, Pb) in seeds [21].

Mihalache et al. [22] investigated the translocation of metals (copper, zinc, lead, cobalt, nickel, manganese, chromium, cadmium) in different organs of various vegetal species (tomato, lettuce, sugar beet, soy, oats, maize) grown on soil amended with 30 t/ha sewage sludge. The results show an important variability of the metals' concentrations in different plant organs. The most tolerant specie according to tolerance index is lettuce (1.70), followed by maize (1.47), soy (1.39), oats (1.09), tomatoes (1.07), sugar beet (0.94).

The fertility benefits of sewage sludge application can be evidenced against the potential hazardous effects of metal accumulation by evaluating the plants sensitivity at various sewage sludge doses. Moreover, because heavy metal pollution is increasing, it is irreversible and has long lasting character, application of sewage sludge must be monitored strictly and environmental risk produced by metals is mandatory to be estimated.

Therefore, the purpose of the present study was to evaluate the suitability use of sewage sludge for sunflower

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a factor =sewage sludge doses	b factor = fertilizer type applied
a_1 - unfertilized with sewage sludge	b_1 – sewage sludge ($N_0P_0K_0$ - without mineral fertilization)
a_2 - fertilization with sewage sludge corresponding to 100 kg N/ha	b_2 – sewage sludge and mineral fertilizer $N_{100}P_{100}K_{100}$ - fertilization 100 kg N/ha + 100 kg P/ha + 100 kg K/ha
a_3 - fertilization with sewage sludge corresponding to 200 kg N/ha	
a_4 - fertilization with sewage sludge corresponding to 300 kg N/ha	
a_5 - fertilization with sewage sludge corresponding to 400 kg N/ha	
a_6 - fertilization with sewage sludge corresponding to 500 kg N/ha	
a_7 - fertilization with sewage sludge corresponding to 600 kg N/ha	

Table 1
DESCRIPTION OF BIFACTORIAL EXPERIMENTAL MODEL

(*Helianthus annuus* L.) by evaluating the growth and yield responses and also, macronutrients and heavy metals accumulation. There were used different sewage sludge rates associated or not with mineral fertilization.

In the last years, sunflower (*Helianthus annuus* L.) gained growing interest for phytoremediation purposes taking into account its' high tolerance to heavy metals [23-27] and as source of vegetable oil and biomass supplier [28]. Moreover, it seems that it is the most promising terrestrial candidate for metal and radionuclides removal from water by rhizofiltration [29].

The experimental developed model was a bifactorial one (7x2) and the studied factors were: A factor – sewage sludge fertilization with seven degrees representing equivalent doses varying between 0-600 kg/ha and B factor – mineral fertilization (NPK) with two degrees: b_1 – $N_0P_0K_0$ and b_2 – $N_{100}P_{100}K_{100}$.

Experimental part

Experimental design

It was developed a bifactorial experimental model (table 1), fully described and characterized previously [30].

For experimental model there were used 56 vegetation pots (20 L capacity), each of 14 treatments in four repetitions. For experiments was used soil collected from A_0 horizon of luvisol meanwhile sewage sludge that was used in experiment was collected from the Wastewater Treatment Plant (WWTP) from Piteşti.

Soil and sewage sludge detailed chemical characterization were reported in a previous paper [30].

In order to study the influence of sewage sludge application on crop quality and yield, in experimental pots were used as test plant sunflower (*Helianthus annuus*L.). After germination, sunflower plants were thinned to three in each pot. Identical water regime was maintained for all pots throughout the growth period of the plants.

Plant sampling and chemical analysis

Plants were taken to the laboratory and it was measured the height of each sample. Then it was partitioned for analysis into stem, leaves, calatidium. All these fractions were washed with distilled water to remove soil particles and oven dried at 70°C until constant weight was achieved.The plants were weighted for biomass measurements.

For macronutrients and heavy metals analysis, 1 g of grounded sample passed through a sieve of 2 mm mesh size was digested with 10 mL from a mixture of acids (HNO_3:H_2SO_4:$HClO_4$, 5:1:1) till a transparent colour appeared [31].

Total nitrogen content was evaluated using Kjeldahl method, meanwhile total phosphorus was assessed spectrophotometrically (as molybdenum blue) and potassium using flame photometry method.

The other metals that presented importance in this research (Ca, Mg, Mn, Ni, Cu, Zn, Cd, Pb) were determined after filtering the digested samples through atomic absorption spectrometry technique.

Results and discussions

Biomass measurements

Sewage sludge fertilization produces significant increase of stem and leaves mass (table 2); significant increases are evident starting with 200 kg N/ha dose, the highest values being obtained in the case of 600 kg N/ha applied dose. Mineral fertilization produces significant increases of the mass.

Association between sewage sludge and mineral fertilizers did not exhibit significant increases of the stem and leaves mass in comparison with sewage fertilization solely.

Significant increases of calatidium mass appear in the case of 200 kg N/ha dose, meanwhile the highest values are registered in the case of 600 kg N/ha sewage sludge dose. Mineral fertilization did not produced significant changes of calatidium mass in comparison with mineral unfertilized variants.

The results concerning sunflower plants height revealed that significant differences appeared only after sewage doses higher than 400 kg N/ha. The largest height of the plants was encountered in the case of 600 kg N/ha dose of sewage sludge. Mineral fertilization ($N_{100}P_{100}K_{100}$) did not lead to significant height growth of the plants in comparison with those mineral unfertilized ($N_0P_0K_0$).

Parameter / Sewage sludge		Mass, g						Height, cm		
		stem+leaves			calatidium					
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kgN/ha	39	42	41a*	31	35	33a*	138	139	139a*
a_2	100 kgN/ha	42	45	44ab	32	37	35ab	138	139	139a
a_3	200 kgN/ha	46	50	48abc	32	39	36abc	141	141	141a
a_4	300 kgN/ha	48	55	52bcd	40	41	41bc	141	143	142a
a_5	400 kgN/ha	50	56	53cd	41	43	42bcd	145	146	146ab
a_6	500 kgN/ha	52	56	54cd	43	43	43cd	151	152	152bc
a_7	600 kgN/ha	57	38	58d	48	50	49d	161	160	161c
Av.(a)		48a*	52b	-	38a*	41a	-	145a*	146a	-

Table 2
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON MASS AND PLANT HEIGHT

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

N, P, K, % / Sewage sludge		N			P			K		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kgN/ha	1.73	1.77	1.75a*	0.49	0.40	0.45a*	2.39	1.08	1.04*
a_2	100 kgN/ha	1.98	2.00	1.99a	0.45	0.37	0.41a	2.42	2.46	2.44a
a_3	200 kgN/ha	2.35	2.45	2.40b	0.48	0.39	0.44a	2.27	2.39	2.33a
a_4	300 kgN/ha	2.42	2.47	2.45b	0.38	0.34	0.36a	2.43	2.63	2.53a
a_5	400 kgN/ha	2.42	2.54	2.48b	0.38	0.37	0.38a	2.54	2.72	2.63a
a_6	500 kgN/ha	2.61	2.78	2.70b	0.38	0.40	0.39a	2.42	2.61	2.52a
a_7	600 kgN/ha	2.47	2.79	2.63b	0.44	0.40	0.42a	2.51	2.65	2.58a
Av.(a)		2.28a*	2.40b	-	0.43a*	0.38a	-	2.43a*	2.55a	-

Table 3
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON N, P, K CONTENTS IN CALATIDIUM

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Sewage sludge application associated with mineral fertilizers did not produced significant increases of the plants' height in comparison with sewage fertilization.

Macronutrients accumulation

Nitrogen content

Nitrogen content in calatidium increased significant after sewage sludge fertilization with doses higher than 200 kg N/ha, the highest values being observed for 500 kg N/ha dose. Mineral fertilization $N_{100}P_{100}K_{100}$ indicates statistic significant increase of nitrogen in calatidium in comparison with those mineral unfertilized.

The lowest level of nitrogen in calatidium was obtained for variants where it was not applied sewage sludge, meanwhile the highest concentrations were obtained in the case of a dose equivalent with 600 kg N/ha in variant with mineral fertilization ($N_{100}P_{100}K_{100}$).

The great increase of nitrogen in calatidium in the case of sewage sludge fertilization indicates that sunflower plants assimilate the nitrogen from organic fertilizer.

Nitrogen content in sunflower seeds found by Murillo et al. [32] is 2.81% in the case of plants grown on soil affected by sludge from Guadiamar river.

Phosphorus content

Phosphorus content in calatidium presents a great variability and it is not significantly influenced by fertilization with sewage sludge, being observed even a downward trend.

Organic and mineral combined fertilization variants did not conducted to significant changes of phosphorus content in calatidium.

Similar phosphorus average level (0.51%) was found by Murillo et al. [32] in the case of sunflower seeds grown spill-affected soil.

Potassium content

Application of organic and mineral fertilizers together did not lead to statistically significant changes of potassium in calatidium, but it notes that organic and mineral fertilized variants led to potassium levels that are higher than mineral unfertilized variants.

The lowest concentration of potassium in calatidium were identified in the case of variants were it was applied sewage sludge equivalent with 200 kg N/ha, meanwhile the highest was registered in the case of 400 kg N/ha dose.

Calcium content

Calcium content in stem and leaves increases with sewage sludge dose, the highest concentrations being observed in the case of 500 kg N/ha dose. Also, mineral fertilization by itself and association between mineral and organic fertilizers did not bring significant changes of the calcium content in stem and leaves.

In the case of unfertilized variants there were obtained the lowest values of calcium meanwhile application of sewage sludge in a dose equivalent with 500 kg N/ha provided the highest calcium levels.

Murillo et al. [32] reported 1.67% Ca in stem of sunflower grown on spill-contaminated soil, meanwhile in petioles found 4.96% Ca.

In the case of calatidium, analyses revealed that sewage sludge fertilization led to a downward trend of calcium content.

Magnesium content

Sewage sludge fertilization favours increasing of magnesium levels in stems and leaves, the highest values being observed in the case of a dose equivalent with 500 kg N/ha. Mineral fertilization did not produce significant changes of magnesium levels in stem and leaves in comparison with unfertilized variants.

Ca,% / Sewage sludge		stem+leaves			calatidium		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kgN/ha	1.00	1.08	1.04a*	1.24	1.18	1.21a*
a_2	100 kgN/ha	1.05	1.10	1.08a	1.15	1.02	1.09a
a_3	200 kgN/ha	1.13	1.11	1.12ab	1.02	1.04	1.03a
a_4	300 kgN/ha	1.20	1.18	1.19ab	1.00	1.02	1.01a
a_5	400 kgN/ha	1.24	1.30	1.27ab	1.02	1.11	1.07a
a_6	500 kgN/ha	1.48	1.52	1.50b	1.00	1.03	1.02a
a_7	600 kgN/ha	1.45	1.50	1.48b	1.04	1.06	1.05a
Av.(a)		1.22a*	1.26a	-	1.07a*	1.07a	-

Table 4
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON CALCIUM CONTENT

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Mg,% / Sewage sludge		stem+leaves			calatidium		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kgN/ha	0.70	0.87	0.79a*	0.85	0.72	0.79a*
a_2	100 kgN/ha	0.75	0.85	0.80a	0.79	0.62	0.71a
a_3	200 kgN/ha	0.90	0.92	0.91ab	0.83	0.69	0.76a
a_4	300 kgN/ha	1.01	1.08	1.05bc	0.73	0.65	0.69a
a_5	400 kgN/ha	1.05	1.06	1.06bc	0.85	0.73	0.79a
a_6	500 kgN/ha	1.20	1.17	1.19c	0.77	0.74	0.76a
a_7	600 kgN/ha	1.14	1.14	1.14c	0.76	0.72	0.74a
Av.(a)		0.96a*	1.01a	-	0.80a*	0.70b	-

Table 5
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON MAGNESIUM CONTENT

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

The highest magnesium content and the lowest one in stems and leaves were achieved in the same conditions as calcium.

Sewage sludge fertilization in doses equivalent with 100-600 kg N/ha did not produced significant changes of magnesium in calatidium. Mineral fertilization lead to significant decrease of magnesium, but combined application of organo-mineral fertilizers did not produce significant modification of magnesium in calatidium.

Plant nutrition experiment reveals magnesium contents between 0.59% and 0.70% in leaves of sunflowers cultivated on sewage sludge amended soil from São Paulo State, Brazil [33].

Metals accumulation

Lately, there are researches [24-27] that indicate the potential of sunflower to be used in phytoremediation processes, taking into account its' high tolerance to heavy metals.

Manganese accumulation

Sewage sludge application produces significant increases of manganese in stems and leaves, the highest values being obtained after application a dose equivalent with 500 kg N/ha and the lowest in the case of unfertilized variant. Mineral fertilization and application of mineral fertilizers combined with sewage sludge did not produced significant changes of manganese in plant in comparison with unfertilized variants.

In the case of manganese content in calatidium it was observed that sewage sludge application produces significant increases, the highest values being obtained for variants where it was applied maximum dose of sewage. The lowest concentrations were obtained for unfertilized variants.

Manganese contents found in stems and leaves are higher than in calatidium, this behavior being also reported by Sabudak et al. [34].

Literature studies report that normal levels of manganese in leaves ranges between 15-150 mg/kg (dry weight), meanwhile phytoxic effects appear at concentrations

Mn, mg/kg / Sewage sludge		stem+leaves			calatidium		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kgN/ha	67	104	86a*	74	84	79a*
a_2	100 kgN/ha	88	110	86a*	72	87	80a
a_3	200 kgN/ha	97	109	103ab	74	96	85a
a_4	300 kgN/ha	125	144	135c	74	95	85a
a_5	400 kgN/ha	152	145	149c	77	103	90ab
a_6	500 kgN/ha	157	152	155c	79	100	90ab
a_7	600 kgN/ha	112	108	110b	83	120	102b
Av.(a)		114a*	125a	-	76a*	98b	-

Table 6
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON MANGANESE CONTENT

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Cu, mg/kg / Sewage sludge		stem+leaves			calatidium		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kgN/ha	9.4	9.2	9.3a*	16.0	17.0	16.5a*
a_2	100 kgN/ha	10.7	10.3	10.5ab	20.5	21.1	20.8b
a_3	200 kgN/ha	13.0	11.8	12.4bc	22.8	21.7	22.3b
a_4	300 kgN/ha	13.7	14.3	14.0c	24.3	23.3	23.8bc
a_5	400 kgN/ha	14.2	15.1	14.7cd	25.1	25.7	25.4cd
a_6	500 kgN/ha	16.8	17.6	17.2d	26.7	27.0	26.9d
a_7	600 kgN/ha	16.0	18.2	17.1d	27.5	28.2	27.9d
Av.(a)		13.4*	13.8a	-	23.3a*	23.4a	-

Table 7
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON COPPER CONTENT

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Zn, mg/kg / Sewage sludge		stem+leaves			calatidium		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kgN/ha	73	65	69a*	25	24	25a*
a_2	100 kgN/ha	111	103	107ab	38	34	36ab
a_3	200 kgN/ha	143	135	139bc	46	41	44bc
a_4	300 kgN/ha	162	156	159c	55	56	56c
a_5	400 kgN/ha	308	291	300d	57	55	56c
a_6	500 kgN/ha	326	313	320d	69	75	72d
a_7	600 kgN/ha	405	395	400e	90	102	96e
Av.(a)		218a*	208a	-	54a*	55a	-

Table 8
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON ZINC CONTENT

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Cd, mg/kg / Sewage sludge		stem+leaves			calatidium		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kgN/ha	0.40	0.37	0.39a*	0.35	0.34	0.35a*
a_2	100 kgN/ha	0.40	0.38	0.39a	0.43	0.46	0.45ab
a_3	200 kgN/ha	0.43	0.38	0.41a	0.56	0.63	0.60b
a_4	300 kgN/ha	0.49	0.45	0.47ab	0.65	0.49	0.57b
a_5	400 kgN/ha	0.55	0.55	0.55bc	0.49	0.49	0.49ab
a_6	500 kgN/ha	0.61	0.56	0.59c	0.48	0.42	0.45ab
a_7	600 kgN/ha	0.53	0.56	0.55bc	0.46	0.49	0.48ab
Av.(a)		0.49a*	0.46a	-	0.49a*	0.47a	-

Table 9
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON CADMIUM CONTENT

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

between 400-2000 mg/kg (dry weight) [34]. Anyway, sunflower is recognized as manganese tolerant specie [35].

Copper accumulation

Chemical analyses indicated that sewage sludge fertilization produces increases of copper content in stems and leaves, the found level being below 20 mg/kg; this threshold is considered toxic for sheep. The increasing tendency of copper content in plant after both mineral and organic fertilization is not statistic significantly. In order to avoid copper accumulation in stems and leaves it is recommended to apply sewage sludge in doses below 400 kg N/ha.

Helianthus annuus L. has potential ability to accumulate copper without being overly sensitive to copper toxicity [34].

Copper content in calatidiumis significant increasing after sewage sludge application. The copper levels are higher in calatidium than in stems and leaves, this situation being expectable having in view copper tendency to accumulate in sunflower seeds [36].

Some researchers [34] reported that sunflower seeds accumulate significantly amounts of copper in comparison with other parts of plant. The maximum copper content found in seeds was 13.85 mg/kg.

Zinc accumulation

Sewage sludge application produces rapid increase of zinc content in stems and leaves, the lowest values being observed in the case of unfertilized variants meanwhile the highest ones were obtained for variants with maximum sewage sludge dose. Application of a dose equivalent with 600 kg N/ha produced to concentrations almost eight times higher than in control variants. Mineral fertilization did not provided statistic significant increases. Even if zinc content in stems and leaves are high after mineral fertilization associated with sewage sludge, the increase is mainly due organic fertilization. Also, significant increase of copper and zinc content in plants after sewage sludge application allow the recommendation to apply it on soils with deficiencies generated by the lack of these microelements.

Sewage sludge doses that substantially change zinc content in stem and leaves are equivalent with values higher than 400 kg N/ha, this allowing to use on normal supplied soils doses lower than 300 kg N/ha.

Even if zinc content in plants present great increases after sewage sludge application (doses equivalent with 600 kg N/ha) it was not exceeded the toxic level (500-1500 mg/kg) [34] but after application of 300 kg N/ha dose, the normal level of zinc in leaves is exceeded (15-150 mg/kg) [34].

Zinc content in calatidium is lower than in stems and leaves but present increases after sewage sludge application. The highest values are recorded in the case of variants fertilized with maximum dose of sewage sludge, when the levels are almost four times greater than unfertilized variant. Mineral fertilization does not produce changes of zinc level in calatidium.

Cadmium accumulation

Sewage sludge fertilization produces significant increases of cadmium in stems and leaves at doses higher than 300 kg N/ha but cadmium concentration is below 1 mg/kg. Mineral fertilization did not produced increase of cadmium in plants. Association between sewage sludge and mineral fertilizers does not lead to statistic significant increases, even if it is clearly noticed an accumulation tendency of this metal in plant.

Cadmium levels in calatidium increases after sewage treatments but this increase presents a great variability, the highest increases being obtained in the case of variants

Ni, mg/kg / Sewage sludge		stem+leaves			calatidium		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kgN/ha	4.1	3.4	3.8ab*	19.8	19.9	19.9a*
a_2	100 kgN/ha	4.3	4.5	4.4a	20.5	20.4	20.5a
a_3	200 kgN/ha	4.7	4.3	4.5a	19.4	17.4	18.4ab
a_4	300 kgN/ha	4.6	4.3	4.5a	17.7	15.8	16.8bc
a_5	400 kgN/ha	4.1	3.4	3.8ab	15.6	15.7	15.7c
a_6	500 kgN/ha	3.8	2.6	3.2b	15.2	15.6	15.4c
a_7	600 kgN/ha	3.3	2.8	3.1b	15.0	15.7	15.4c
Av.(a)		4.1a*	3.6a	-	17.6a*	17.2a	-

Table 10
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON NICKEL CONTENT

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Pb, mg/kg / Sewage sludge	stem+leaves		
	b_1	b_2	Av.(b)
0 kgN/ha	3.4	3.3	3.4a*
100 kgN/ha	3.6	3.6	3.6a
200 kgN/ha	4.0	4.2	4.1ab
300 kgN/ha	4.9	4.6	4.8b
400 kgN/ha	5.1	4.6	4.9b
500 kgN/ha	5.0	5.1	5.1b
600 kgN/ha	5.0	5.0	5.0b
Av.(a)	4.4a*	4.3a	-

Table 11
INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON LEAD CONTENT

b_1 corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

fertilized with sewage doses equivalent with 200-300 kg N/ha.

Association between organic and mineral fertilizers did not produced significant changes of cadmium in calatidium.

De Maria et al. [37, 38] found that sunflower grown on contaminated soil accumulate cadmium mainly in root and old leaves with no detrimental effects on plant growth and physiological parameters, excepting chlorophyll content.

Nickel accumulation

Sewage sludge fertilization with small doses (100-300 kg N/ha) produce an increase of nickel in stems and leaves, but at higher doses this behaviour disappear, probably as effects of sewage sludge that decrease nickel mobility. Nickel levels in plant (stems and leaves) are below 5 mg/kg and there are no concerns regarding the quality of the plants.

Mineral fertilization lead to a decrease tendency of nickel, meanwhile association between sewage sludge with mineral fertilization did not conducted to statistic significant increases.

Also, it was observed that sewage sludge produces a significant decrease of nickel in calatidium. When is applied a dose equivalent with 100 kg N/ha, nickel concentration increases in comparison with control variant. Moreover, analyses indicated that nickel content in calatidium is higher than in stems and leaves, this behaviour being reported in literature [39].

Lead accumulation

Lead accumulation in stems and leaves is produced when applied sewage sludge doses are higher than 300 kg N/ha. Average lead concentration is below 5 mg/kg, this permitting to use the sewage sludge as fertilizer.

Common lead concentrations in plants are generally lower than 10 mg/kg and 3 mg/kg is considered a normal lead content [39].

Literature studies [39, 40] suggest that uptake of lead is probably passive and translocation from roots to other plant parts is low. In our study, this behaviour is fully revealed because in calatidium, lead levels were below detection limit of the method.

Conclusions

The research aim was to evaluate the effect of sewage sludge application in various doses (associated or not with mineral fertilizers) on growth of *Helianthus annuus* L. by assessing the biomass measurements, macroelements (N, P, K, Ca, Mg) and potential hazardous metals accumulation (Mn, Cu, Zn, Cd, Ni, Pb).

The results led us to the conclusions that are highlighted below:

- the growth of *Helianthus annuus* L. was well developed on soil amended by sewage sludge application;
- sewage sludge fertilization produces significant increase of biomass and are evident starting with 200 kg N/ha dose;
- nitrogen and potassium contents in calatidium increased with sewage sludge loading, meanwhile in the case of phosphorus has been observed a downward trend;
- calcium and magnesium contents in stem and leaves increase with sewage sludge dose, the highest concentrations being observed at500 kg N/ha dose and in calatidium the organic fertilization led to a downward trend;
- sewage sludge application produces significant increases of manganese, copper, zinc and cadmium in stems and leaves;

- manganese and zinc contents found in stems and leaves are higher than in calatidium, meanwhile in the case of copper and nickel was observed the opposite situation;

- average lead concentration in stem and leaves is below 5 mg/kg, this permitting to use the sewage sludge as fertilizer and lead levels in calatidium were below detection limit of the method;

- fertilization with sewage sludge doses equivalent to 600 kg N/ha did not produce excessive accumulation of metals in sunflower plants and did not led to nutrition disorders;

- arguably sunflower can be fertilized with sewage sludge without the risk of lowering crop quality and there are no concerns regarding environmental issues correlated with high levels of metals in sunflower plants.

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